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Brief Original Article

Detection and quantification of *Campylobacter* spp. in Brazilian poultry processing plants

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Abstract

Introduction: Campylobacteriosis is considered the most common bacteria-caused human gastroenteritis in the world. Poultry is a major reservoir of *Campylobacter*. Human infection may occur by consumption of raw and undercooked poultry or by contamination of other foods by these items. The aim of this study was to assess the prevalence of *Campylobacter* spp. in poultry processing plants with conventional culture method and real-time PCR.

Methodology: A total of 108 poultry processing plant samples were collected to test with conventional microbiology and qPCR. Sampling included cloacal swabs, swabs of transport crates (before and after the cleaning and disinfection process) and carcasses (after the chiller, cooled at 4°C and frozen at −12°C).

Results: Positivity in cloacal swabs indicated that poultry arrived contaminated at the slaughterhouse. Contamination in transport cages was substantially increased after the cleaning process, indicating that the process was ineffective. The detection of *Campylobacter* on carcasses was higher than that on cloacal swabs, which could indicate cross-contamination during the slaughtering process. Conventional microbiology and molecular methods revealed a prevalence of 69.4% and 43.5%, respectively. Lower detection by qPCR can be attributed to the high specificity of the kit and to biological components that could inhibit PCR reactions.

Conclusions: Our results indicate that poultry arrive contaminated at the slaughterhouse and that contamination can increase during the slaughtering process due to cross-contamination. The isolation of *Campylobacter* in cooled and frozen carcasses corroborates the bacterial survival even at temperatures considered limiting to bacterial growth which are routinely used for food preservation.

Key words: qPCR; *Campylobacter*; slaughterhouse; poultry.

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Introduction

Campylobacteriosis is a foodborne disease and is considered the most common bacteria-caused human gastroenteritis in the world. Poultry is a major reservoir and source of transmission of *Campylobacter* species to humans. Human infection may occur by consumption of raw and undercooked poultry or by cross-contamination by these products [1-4]. *Campylobacter* spp. are usually recovered from poultry slaughterhouses, both from processing equipment and from the plant environment. After their introduction into the slaughter line, the bacteria can be spread to poultry meat [5]. Studies evaluating the prevalence of *Campylobacter* spp. in Brazilian poultry meat are still incipient [6-11].

Traditionally, identification methods have been based on the use of selective culture media and biochemical tests. However, *Campylobacter* spp. are fastidious bacteria that may be difficult to recover in culture due to suboptimal specimen transport and/or storage conditions [12]. Therefore, the identification of *Campylobacter* spp. and the differentiation among species through conventional bacteriology techniques are time-consuming and challenging [13]. Thus, molecular biology techniques have been studied as an alternative for the conventional laboratory methods. Frequently employed molecular biology techniques for the identification of *Campylobacter* spp. include PCR (with variations) and sequencing, which are easy and rapid approaches [14].

In this context, the aim of this study was to detect and assess the prevalence of *Campylobacter* spp. in Brazilian processing plants by conventional culture method and by real-time quantitative polymerase chain reaction (qPCR).

Methodology

Samples selection

Three broiler slaughterhouses under the federal or state inspection system from the Rio Grande do Sul state were sampled twice over a period of six months. All samples were collected from female Cobb broilers, at 42 days with average slaughter weight of 2300g. Sampling included cloacal swabs at the reception of the broilers at the slaughterhouses, swabs of transport crates (before and after the cleaning and disinfection process) and carcasses (after the chiller, cooled at 4°C and frozen at −12°C). Samples were collected in triplicate at each establishment. At the end of the sampling period, a total of 108 samples were analyzed.

For the cloacal swabs, six pools per establishment were collected, and each pool included 50 swabs. Each swab was used for two birds, with a total of 300 sampled birds. Birds were randomly selected as soon as the transport crates were removed from the trucks. The swabs were collected at the reception of slaughterhouse and immediately inoculated into 50 mL of Bolton broth. Transport crates were randomly selected and identified with seals for control before being cleaned in an automated washing system. Commercial sponge-sticks with neutralizing buffer (3M®, Maplewood, USA) were used throughout the entire crate extension before and after the cleaning and disinfection process. The sponges were packed in sterile bags with 50 mL of Brucella broth. The carcasses were randomly selected and were stored in individual sterile plastic bags and identified with seals. In the laboratory, each sample was rinsed with 400 mL of buffered peptone water and homogenized for 30 seconds.

Detection of Campylobacter spp. by conventional microbiology

The detection and isolation of *Campylobacter* spp. at genus level was performed according to the criteria described by the International Organization for Standardization [15].

Real-time quantitative PCR (qPCR) analysis

The DNA was thermal extracted using a mericon DNA Bacteria Kit (Qiagen, Manchester, UK). Amplification of *Campylobacter* spp. was performed with the mericon *Campylobacter* triple Kit (Qiagen, Manchester, UK), designed for the target-specific detection of *C. jejuni, C. coli,* and *C. lari*, in a Rotor-Gene real-time PCR system (Qiagen, Manchester, UK). The reactions included a total volume of 20 µL containing 10 µL of reconstituted mericon assay master mix and 10 µL of genomic DNA. Amplification was carried out at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 10 seconds using a Rotor-Gene real-time PCR system.

Statistical analysis

The data obtained were subjected to statistical analysis using PASW Statistics 18.0.0 (IBM; Hong Kong) software. Fisher's exact test was used to compare *Campylobacter* detection before and after the cleaning and disinfection process and to compare the detection in poultry carcasses throughout processing. ANOVA was used to compare qPCR quantification means. Chi-square (χ^2) test was used to determine differences between the applied techniques for Campylobacter detection. Significance was defined as $p < 0.05$.

Results

The results, according to the source of isolation, are described in Table 1.

Campylobacter spp. prevalence did not present a difference ($p > 0.05$) among the three slaughter plants where the bacteria were identified, independent of the technique of detection. The frequency of *Campylobacter* spp. detection varied significantly ($p < 0.05$) between the two techniques. The conventional microbiology method and the molecular method presented a prevalence of 69.4% (75/108) and 43.5% (47/108), respectively.

Table 1. Prevalence of *Campylobacter* spp. detected by conventional microbiology methods and real-time quantitative PCR (qPCR) for each source of isolation.

Source of isolation	**Prevalence % (n/N)**	
	Conventional microbiology	**qPCR**
cloacal swab	44.4 (8/18)	88.9 (16/18)
swabs of transport cages (before cleaning)	50.0 (9/18)	38.9 (7/18)
swabs of transport cages (after cleaning)	83.3 (15/18)	83.3 (15/18)
carcasses after the chiller	66.7 (12/18)	22.2 (4/18)
carcasses cooled at 4°C	88.9 (16/18)	22.2 (4/18)
carcasses frozen at −12°C	83.3 (15/18)	11.1 (2/18)

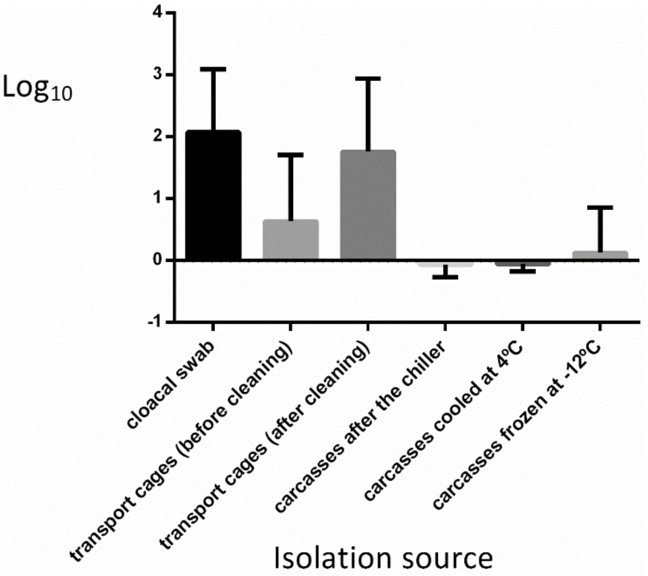
There were no differences ($p > 0.05$) in *Campylobacter* spp. detection between the swabs of transport cages taken before and after the cleaning process or among the carcasses after the chiller, cooled at 4°C or frozen at −12°C, regardless of the detection technique.

The results for qPCR quantification varied according to the source of isolation and are showed in Figure 1. The highest quantification was found in cloacal swab (2.1), followed by swabs of transport cages (after cleaning) (1.8) and swabs of transport cages (before cleaning) (0.6). The lowest levels were found in carcasses collected after the chiller (0.0), cooled at 4ºC (0.0) and frozen at -12ºC (0.1). The cloacal swab quantification as determined by qPCR showed a significant difference ($p < 0.05$) from that of all other sources, except for that of the swabs of transport cages taken after the cleaning process. There was a difference ($p < 0.05$) in the bacterial load in the transport cages before and after the cleaning and disinfection processes. The bacterial loads in the swabs of the transport cages taken before cleaning also differed ($p < 0.05$) from those of the carcasses cooled at 4°C, and the bacterial loads in the swabs of transport cages taken after cleaning differed ($p < 0.05$) from those of all carcasses, independent of the source of isolation. There were no significant differences ($p > 0.05$) in bacterial loads among the carcasses after the chiller, cooled at 4°C and frozen at −12°C.

Discussion

Campylobacter occurrence in poultry meat is variable depending on the country or region. In Brazil, it varies from 4% to 88% in poultry sources [6-11]. Brazilian data about *Campylobacter* human infection are still insufficient. Some problems favor the underdiagnosis and underreporting of campylobacteriosis, such as the difficulty of isolation and the expensive supplies that are necessary for bacterial cultivation. Unlike United States and European countries, in Brazil there is no specific legislation for the analysis of *Campylobacter*. However, it would be useful if the Brazilian Ministry of Agriculture, Livestock and Supply expand the research in this area to better analyze the occurrence of this pathogen in products of animal origin. In United States, for example, Campylobacteriosis was not nationally notifiable until 2015, and even if little national information is available, these data provide baseline rates for monitoring changes and it is useful for the elucidation of newly aspects of *Campylobacter* epidemiology in this country [16]. The absence of an internal monitoring program adopted by Brazilian poultry companies may justify the lack of national data [17]. However, the Ministry of Agriculture recently conducted an exploratory program for monitoring *Campylobacter* spp. in poultry meat between July 2017 and July 2018, but the results have not yet been released (data not shown).

Figure 1. Quantification of Campylobacter spp. through real-time quantitative PCR for each source of isolation.



Although the processing plant environment presents harsh conditions for microorganism survival [5], *Campylobacter* was detected in all sources of isolation in the three different slaughter plants. In this study, almost 90% of the samples from cloacal swabs were positive as assessed by qPCR. Positivity in cloacal swabs indicates that the poultry arrives contaminated at the slaughterhouse. It is likely that chickens arriving contaminated in the slaughterhouse were contaminated on the farm. According to Hermans *et al*. [18], there is a probability of 60% to 80% for a flock to be positive for *Campylobacter* at slaughter age. Several factors have been associated with *Campylobacter* infection on the farm, including the flock age, number of days between flocks, positive status of the previous flock, presence of rodents, flies, wild animals, domestic animals, personnel, equipment, water and feed [19]. Moreover, a significant correlation between the contamination of the broilers during rearing and the carcasses after processing has been shown [20]. In this context, it is important to avoid cross-contamination of carcasses during the slaughter process. However, according to Herman *et al*. [19], slaughterhouses could not avoid contamination of carcasses when a flock is positive for the presence of *Campylobacter*. Similarly, in the present study, the cleaning and disinfection process seemed to be ineffective, as *Campylobacter* contamination in the transport crates was substantially increased after this. Crates are a suitable reservoir for *Campylobacter* spp. and represent a risk of infection for uninfected birds [21]. They are considered a potential source of *Campylobacter* for negative flocks since they are reused [22]. According to Peyrat *et al*. [23], the cleaning and disinfection process of equipment and crates in poultry processing plants is not effective and may contaminate carcasses during the slaughter process.

Carcasses at all stages of processing presented high contamination. The amount of *Campylobacter* detected on carcasses was higher than the prevalence in cloacal swabs, which could indicate cross-contamination during the slaughtering process. Carcass contamination can occur throughout the entire slaughtering process, with major points of cross-contamination on the slaughter line [19]. It most frequently occurs during the evisceration operation, as the rupture of viscera can release high numbers of *Campylobacter* cells onto the carcass [24]. Cross-contamination of carcasses occurs by the contact of negative flocks' carcasses with surfaces and utensils contaminated by positive flocks' carcasses [19]. On the other hand, *Campylobacter* quantification by qPCR indicated a lower concentration in the carcasses, indicating a reduction in amount of cells. In addition, an increase in *Campylobacter* detection was observed when the frequency in the carcasses after the chiller was compared with the frequency in cold or frozen carcasses. These data are of concern because *Campylobacter* is able to survive for long periods on food processing equipment surfaces, even after cleaning and disinfection procedures [23]. The isolation of *Campylobacter* in cooled and frozen carcasses corroborates the bacterial survival characteristics in humid environments even at temperatures considered limiting to bacterial growth, such as 4°C and −12°C, which are routinely used for food preservation.

The frequency of *Campylobacter* spp. detection varied between conventional microbiology and the molecular method. Lower detection by qPCR can be attributed to the high specificity of the kit, which is designed for the detection of thermophilic species and thus may not amplify other species. Further studies testing different primers are needed to improve qPCR analysis. Also, it is possible that samples presented components that are potentially inhibitory to PCR reactions, such as biological materials (feces, blood and fat) and active ingredients used in the cleaning and disinfection process, including chlorine, of slaughter plants and equipment [25]. On the other hand, qPCR detected positive samples that were determined to be negative by the conventional microbiology methods. Even if *Campylobacter* can enter a "viable but not culturable" state that is difficult to detect with conventional methods and which could difficult its detection, this discrepancy is more likely related to the detection of free Campylobacter DNA. Preventive approaches such good hygiene practices and biosecurity could be a strategy to prevent the colonization of animals by *Campylobacter* and to control this agent in the poultry production chain [26]. The development of indirect measures, complementary of best practices, can reduce the intestinal number of Campylobacter in poultry. This information was added to the revised manuscript.

Conclusion

Our results indicate that, in the three evaluated establishments, poultry arrive contaminated at the slaughterhouse and that contamination can increase during the slaughtering process due to cross-contamination. The isolation of *Campylobacter* in cooled and frozen carcasses corroborates the bacterial survival characteristics in humid environments even at temperatures considered limiting to bacterial growth, such as 4°C and −12°C, which are routinely used for food preservation.

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